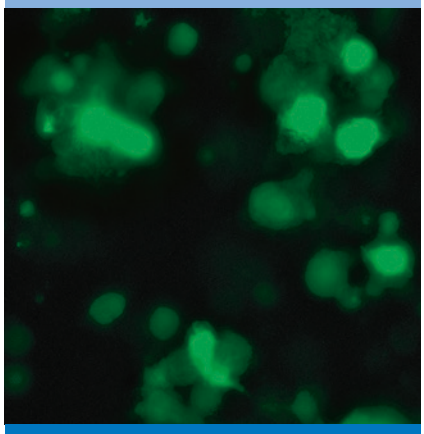
Lonza

Nucleofector™ Technology – Combining High Transfection Performance with Superior Preservation of Functionality



Abstract

Nucleofection™ has become a method of choice whenever transfection of primary cells or difficult-to-transfect cell lines is required. Here we show that Nucleofection™ of frequently used primary cells (mouse dendritic cells, human macrophages and human T cells) results in highly efficient transfer of DNA and other substrates, while at the same time maintaining excellent cell viability and post-transfection functionality. This combination of benefits makes Nucleofection™ superior to other transfection methods.

Introduction

Transient transfection of primary cells can be achieved by lipofection, electroporation and various other methods. However, none of these classical transfection methods gain high transfection rates combined with low post-transfection mortality and a good preservation of cell specific functionality.

With the Nucleofector™ Technology, Lonza offers a non-viral, easy-to-use transfection method unifying these three aspects. It is the combination of dedicated cell type-specific Nucleofector™ Solutions together with optimized and unique electrical parameters for each cell type that enable such an outstanding performance. To further minimize the effects of the transfection process on cell functionality, great care is taken during Lonza's optimization of the Nucleofection™ Parameters. This includes for example the verification of cellular functionality utilizing frequently used cell-based assays early in the developmental process. This short report focuses on three frequently used, difficult-to-transfect primary cells (mouse dendritic cells, human macrophages and human T cells), which proves the high percentage of post-transfection functionality that can so far only be reached using Nucleofection™.

Dendritic cells play a central role in the mammalian immune system. Their main function is to process antigen material and present it on their surface to other cells of the immune system. Once activated by contact with a pathogen, dendritic cells mature and grow branched projections, the dendrites that give the cell its name. In parallel, the activated cells start to express typical immune response molecules such as Interleukin-6. Mouse dendritic cells are a frequently used model system to elucidate the functionality of these antigen-presenting cells within the complex mammalian immune system. Performing such studies requires an efficient transfection method that does not alter the immunological functionality. Apart from Nucleofection™, no other efficient and easy-to-use transfection system is available for these cells. Nucleofection™ Protocols for immature and mature mouse dendritic cells are available for the different Nucleofection™ Platforms. The 20 µl Nucleocuvette™ Strips used in the 4D-Nucleofector™ or 96-well Shuttle™ System are suitable to transfect low cell numbers (down to 2.5×10^4 mature cells per sample), saving precious animal resources.

Macrophages derive from monocytes upon induction with certain cytokines. Macrophages are active in both innate immunity, as well as adaptive immunity of vertebrates. Their role is to engulf and digest pathogens and cellular debris. Once the digestion of the intruder is finalized, macrophages present the pathogenic antigens on their surface thus stimulating other immune cells to attack the pathogen. A typical marker for biochemically active macrophages is the secretion of Tumor Necrosis Factor Alpha (TNF-α) after contact with a pathogen or pathogen components like lipopolysaccharides (LPS). Hence, studying functionality of primary human macrophages by gain and loss of function studies is of great interest for pharmacologists, immunologists and cancer research scientists.

Besides Nucleofection™, so far no other transfection system is suited to transfect molecules into the nucleus of macrophages with high performance and excellent preservation of cell functionality, making Nucleofection™ a perfect tool for such studies.

T cells play a central role in cell-mediated immunity. They originate from hematopoietic stem cells in the bone marrow and finally mature to thymocytes that are released from the thymus to peripheral tissues afterwards. Activation of T cells occurs through the involvement of the T cell receptor and CD28, both presented on the T cell surface. *In vitro* anti CD3/CD28 antibodies or other compounds are frequently used to induce the immune response of T cells. Upon stimulation, human T cells present the interleukin-2 receptor (IL-2R) on their surface and start to secrete Interferon gamma (IFN-γ). Human T cells isolated from peripheral blood are a model system to study biochemical pathways by loss and gain of function studies utilizing the transfection of either DNA or siRNA molecules. Non-viral transfection of these cells was a major challenge before the release of dedicated Nucleofection™ Protocols and Kits.

Results and Discussion

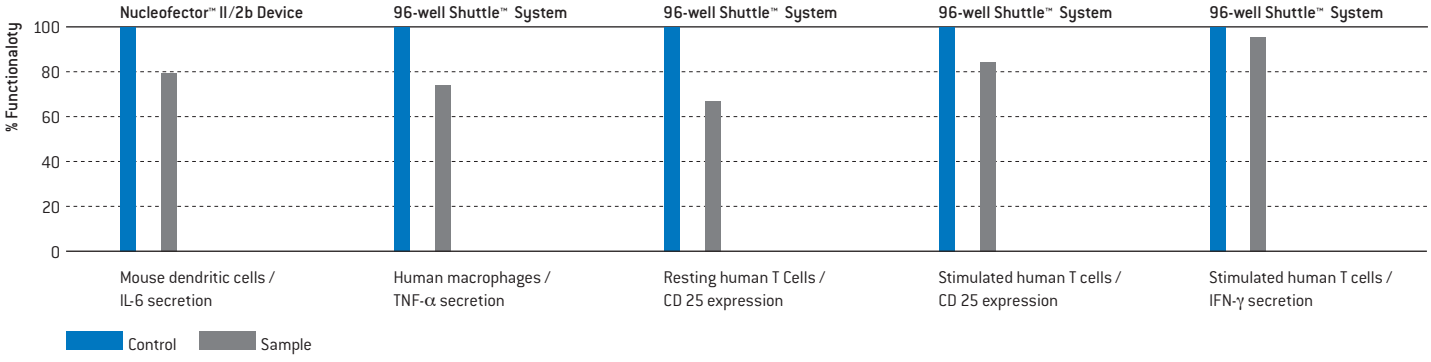
The experiments presented in this article have either been performed on the basic Nucleofector™ II/2b Device (mouse dendritic cells) or the high-throughput 96-well Shuttle™ System (human macrophages and human T cells) using the individual primary cell type-specific kits and protocols. Each primary cell specific kit has been highly optimized by Lonza's team of R&D scientists. This resulted in excellent transfection efficiencies while maintaining cell functionality. Cells were transfected with pmaxGFP™ Vector using the parameters carefully optimized for the dedicated primary cell and analyzed 24 to 48 hours post Nucleofection™ by flow cytometry for GFP expression. Viability was either measured by propidium iodide (PI) staining or using microtiter plate based cell viability assays (CellTiter-GLO®, Promega). Viability values are given in percent compared to the non-transfected control (cells handled as described in the Optimized Protocol but not treated with DNA or Nucleofection™ Program). Functionality of the cells post Nucleofection™ was tested by frequently used cell specific assays as described below.

Table 1 Average transfection efficiency and viability 24 hours post Nucleofection™

Cell Name	Efficiency	Viability	Nucleofector™ II/2b Kit	4D-Nucleofector™ or 96-well Shuttle™ Kit
Dendritic cell, mouse (immature)	34–58%	37–62%	Mouse Dendritic Cell	P4
Dendritic cell, mouse (mature)	29–49%	63–88%	Mouse Dendritic Cell	P3
Macrophage, human	42–59%	60–88%	Human Macrophage	P3
T cell, human (stim.)	41–70%	59–90%	Human T Cell	P3
T cell, human (unstim.)	43–98%	51–92%	Human T Cell	P3

Result ranges represent an extrapolation from larger result collections, including Lonza and customer data. For results from single experiments or from other cell types please refer to the comprehensive at **www.lonza.com/celldatabase**.

Figure 1 Cell functionality post Nucleofection™



Functionality was analyzed post Nucleofection™ by measuring secretion of cytokines (via ELISA) specific for the different cell types: mouse dendritic cells (IL-6), human macrophages (TNF-α), or stimulated human T cells (IFN-γ). In addition, both resting and stimulated human T cell were analyzed by flow cytometry using a CD25 specific antibody. The bars display the relative functionality of the transfected cells (Sample) in percent related to non-transfected control (Control). Experiments were performed on different Nucleofection™ Platforms.

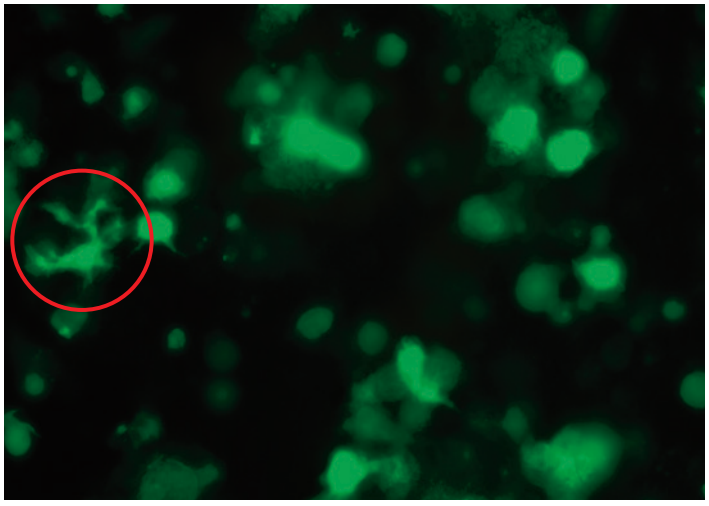
Mouse Dendritic Cells

Nucleofection™ of immature mouse dendritic cells (BALB/c) using the Nucleofector™ II/2b Device resulted in high transfection efficiency (59%) and high cell viability (93%). In parallel, the optimized parameters enabled an excellent preservation of functionality, tested by the ability of the transfected by Nucleofection™ cells to secret IL-6 (Figure 1) after induction with LPS. For this purpose, immature mouse dendritic cells were incubated with LPS 2 hours post Nucleofection™. IL-6 secretion was analyzed by a standard sandwich ELISA (IL-6 ELISA ms, Biosource™) and is given in percent compared to the non-transfected control. In addition, the transfected cells were able to form dendrites as shown in Figure 2.

Human Macrophages

Nucleofection™ of human macrophages using the 96-well Shuttle™ System revealed high efficiency (42%) combined with low mortality. Functionality of human macrophages has been tested by measuring the secretion of TNF-α triggered by stimulation of the cells with LPS 24 hours post Nucleofection™. Data shown in Figure 2 prove the excellent conservation of macrophage functionality post Nucleofection™. TNF-α secretion was analyzed by sandwich ELISA (TNF-α EASIA, Biosource™) and is shown in comparison to non-transfected control (macrophages handled as described in the Optimized Protocol but not treated with DNA).

Figure 2 Mature mouse dendritic cells form dendrites post Nucleofection™.



Immature mouse dendritic cells were transfected by Nucleofection™ with pmaxGFP™ Vector. 2 hours post Nucleofection™, cells were stimulated with LPS to mature. Cells were analyzed 24 hours post Nucleofection™ by fluorescence microscopy for GFP expression and their ability to form dendrites (red circle).

Human T Cells

Human T cells were transfected using 96-well Shuttle™ System according to the respective Optimized Protocols. For unstimulated T cells either program FI-115 (for highest efficiency) or program EO-115 (for highest functionality) was applied. Stimulated T cells were transfected using program EO-115 only. Preservation of the biochemical functionality of resting T cells after Nucleofection™ was analyzed by the detection of interleukin receptor 2 (IL-2R alpha chain or CD25). Therefore, resting human T cells were stimulated with anti-CD3 and anti-CD28 antibodies 5 hours post Nucleofection™. For stimulated cells, maintenance of the stimulated state was analyzed by the expression of CD25 as well as the secretion of Interferon gamma (IFN-γ). Human T cells expressing the surface bound receptor were detected with labeled anti CD25 antibodies (PE Mouse Anti-Human CD25; BD Pharmingen™) by flow cytometry. Secretion of IFN-γ was analyzed by a sandwich ELISA (IFN-γ EASIA, Biosource™). Data (Figure 2) are given in percent compared to non-transfected control.

Summary

Lonza's Nucleofector™ Technology addresses a broad spectrum of cell types, substrates and cell numbers relevant for various research areas, e.g. immunology. With more than 50 years of combined laboratory experience, Lonza's team of scientists optimizes Nucleofection™ Conditions to reach excellent transfection performance combined with superior preservation of cell characteristics, as proven by the examples in this application note and more than 300 publications about T cells, macrophages and dendritic cells cited in Lonza's Nucleofection™ Citation Database (**www.lonza.com/nucleofection-citations**).

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